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The use of tryptic enzyme activity measurement as a nutritional condition index: laboratory calibration data and field application

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Tryptic enzyme activity of herring (*Clupea harengus*), turbot (*Scophthalmus maximus*), and cod (*Gadus morhua*) larvae kept under defined laboratory conditions was determined. Tryptic enzyme activity was related to larval age, length, days of food deprivation, and feeding time. From 10 days after hatching onwards, significant differences in tryptic enzyme activity appeared when larvae were deprived of food for between 3 and 9 d. Diurnal feeding patterns were monitored by measuring individual tryptic enzyme activity. In short-term feeding, starving and re-feeding experiments, tryptic enzyme activity reflects the digestion processes in relation to food ingestion and re-establishment of tryptic enzyme level within hours of re-feeding. Individual tryptic enzyme activity levels in herring and sprat larvae (*Sprattus sprattus*) were determined in field samples and compared with laboratory calibration data in order to evaluate the nutritional condition of the field collected larvae from different sampling sites and different seasons. Continuous sampling of sardine larvae (*Sardina pilchardus*) on an oceanic drift station was used to show diurnal feeding rhythm by applying tryptic enzyme activity as an indicator.

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Introduction

Survival rates and growth of fish larvae depend mainly on the availability of food and its quality in the field or in aquaculture facilities. In field research, mortality rates of fish larvae have been found to be very high; typically, less than 0.01% of larvae hatched reach adulthood. Since this high mortality is regarded to be due to starvation or predation, there has been a need to develop tools with which to determine either nutritional condition or predation rates in the field, thus identifying the major factor determining survival of the early ontogenetic stages of teleosts (Hunter, 1976; Houde, 1987; Bailey and Houde, 1989). This aim provided the background for establishing suitable methods on the basis of biochemical or physiological indicators, methods which could be used to determine larval condition in field samples. Among these (e.g., RNA/DNA ratio, lipids, C/N ratio, histology), tryptic enzyme activity has been suggested as an appropriate indicator of the fitness and survival potential of fish larvae (Hjelmeland *et al.* 1984). Tryptic-like enzymes are universally abundant among marine organisms and are present in a measurable amount even in the youngest stages of fish larvae and in their potential food organisms. Trypsin has been investigated in relation to feeding regimes in a number of studies and has been demonstrated to be a useful indicator of digestive processes and nutritional condition in fish larvae (e.g., Lauff and Hofer, 1984; Pedersen *et al.*, 1987, 1990; Ueberschär, 1988; Ueberschär and Clemmesen, 1992; Ueberschär *et al.*, 1992). In aquaculture there is also a need to evaluate the quality and quantity of food, the feeding frequencies required and digestibility of food achievable in the early stages of marine fish larvae. Although tryptic enzyme activity measurements have been initially applied to basic fisheries research in the field in order to study nutritional aspects related to recruitment problems, this methodological approach to larval condition can also be applied to aquaculture research.

The usefulness of proteolytic enzyme activity measurements in larval fish research has been discussed briefly in Ueberschär (1993). The present study provides a more detailed view on the practical application of tryptic enzyme activity methodology in small-scale laboratory experiments as well as in large-scale field

studies. Results obtained with cod, turbot, and herring larvae kept under well-defined laboratory conditions are presented and show characteristics of feeding quality and quantity in relation to tryptic enzyme activity, with special regard to diurnal patterns. In large-scale field studies tryptic enzyme activity was measured on sprat and sardine larvae in order to study basic aspects of the year-class variations of marine fish species. Some findings from these studies are presented and discussed here.

Materials and methods

Laboratory-raised larvae

Adult herring (*Clupea harengus*) were captured in the Kiel Canal, a well-known spawning site for Baltic herring, in spring 1991 and stripped. The fertilized eggs were incubated on glass plates (400 cm^2 each) at 9.2°C and a salinity of 15 to 16 in aerated glass aquaria (25 l) with a daily water (UV-treated and filtered) exchange of 90% of the volume. After hatching, larvae were transferred to 60 l or 400 l green cubic tanks in a "flow-through" system. The temperature varied between 12.0 and 16.7°C (mean 14.4°C), increasing in parallel with ambient temperatures during the rearing experiments. Herring larvae were fed exclusively with rotifers (*Brachionus plicatilis*) from hatching to day 19 and then supplemented with *Artemia* sp. nauplii from day 19 until the end of the experiments (day 65). Mean concentrations of 5.0 ml^{-1} *Brachionus* and 1.0 ml^{-1} *Artemia* nauplii were adjusted daily in the rearing tanks. Subsequently, larvae were deprived of food for intervals of 1 to 9 days prior to sampling for enzyme measurements. Samples of fed and starved herring larvae were taken in different experimental series in the range of hours to days prior to and after feeding.

Turbot eggs (*Scophthalmus maximus*) were obtained from a commercial local hatchery (BUTT Company) and larvae were reared in the same experimental facilities as herring larvae with increasing temperature during experiments from 15.1 to 19.0°C. The larvae were fed on *B. plicatilis* until day 16 after hatching and *Artemia* sp. nauplii were offered from day 11 onward until the end of the experiment. Starvation intervals ranged from 2 to 6 days for the different experimental series with various ages. In order to investigate diurnal pattern in enzyme activity, samples of fed and starved turbot larvae were taken for different experiments at intervals before and after feeding.

Cod (*Gadus morhua*) larvae from a Baltic stock were reared in the facilities of the BUTT Company under similar conditions as described for herring and turbot and fed with enriched (DHA-Selco) *B. plicatilis* and supplemented with enriched *Artemia* sp. nauplii (DHA-Selco), but kept at lower temperature (6 to 10°C) and a salinity of 20.

The larvae sampled from all laboratory rearing experiments were preserved at −74°C in a deep-freezer. Prior to analysis, the larvae were examined for gut content, and standard length (SL) was measured (precision 0.5 mm).

Field samples

Herring larvae were caught during routine ICES cruises in the English Channel in January 1986 and in the Irish Sea in October 1985 with a MOCNESS system (1 m^2, Wiebe *et al.*, 1976). Sprat larvae from batch-spawning adults were caught on five cruises in the German Bight from May to August 1991 with an HAI system (high-speed plankton sampler ≅ GULF-II type sampler). Sardine (*Sardina pilchardus*) were caught during a cruise off the northwest Spanish coast 1991 with an HAI system. All larvae were separated individually from plankton samples and immediately transferred to Eppendorf® caps, shock-frozen in liquid nitrogen and stored at −74°C in a freezer. Prior to analysis, larvae were examined for damages, gut content was noted, and standard length was measured (precision 0.5 mm). Larvae with damaged guts were discarded.

Measurement of tryptic enzyme activity

Tryptic enzyme activity was assayed in accordance with the fluorescence technique described by Ueberschär (1988), with the following modifications: the temperature was adjusted to 30°C in the measuring system instead of 25°C. After temperature equilibration, 500 µl of the substrate (0.20 mmol Nα-benzoyl-L-arginin-methyl-coumarinylamide, MCA, in TRIS-HCL buffer, 0.1 mol, pH 8.00) was added to 100 µl of the homogenate in the cuvette and mixed well.

Larvae were individually homogenized in 250 µl or 500 µl TRIS-HCL buffer (0.1 mol, pH 8.00), depending on the size of the larva; small larvae were homogenized in a smaller volume of buffer to gain higher enzyme concentrations and to promote fluorescence signals. When necessary, homogenates from large larvae with high enzyme activities were pre-diluted 2 to 10 times before 100 µl was added to the substrate.

The relative fluorescence enhancement (excitation 380 nm, emission 440 nm) was recorded every 2 min over a maximum period of 10 min, using a KONTRON SFM25 spectral fluorometer with a computerized cuvette holding unit. The resulting tryptic enzyme activity per larva is given as the amount of hydrolysed substrate per time unit (hydrolysed MCA $larva^{-1}$ min^{-1}).

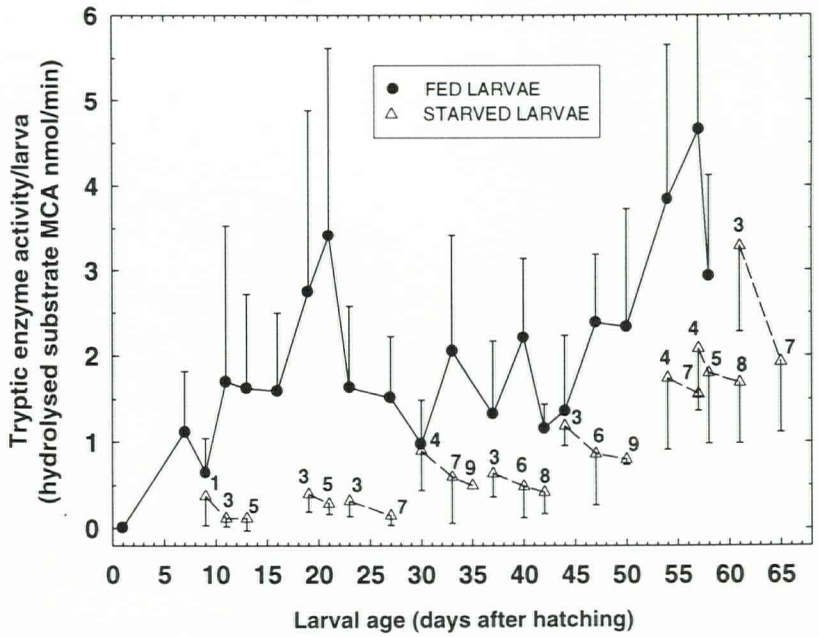


Figure 1. Tryptic enzyme activity in fed (*Brachionus* 5 ml^{-1}, *Artemia* 1 ml^{-1}) and starved herring larvae (*Clupea harengus* L.) reared in the laboratory in relation to larval age. Data points are means of 5 to 15 individually measured larvae; positive error bars are shown (SD = Standard Deviation). The numbers indicate the day larvae were deprived of food prior to sampling.

Results

Reared larvae

Tryptic enzyme activity in reared 1- to 65-day-old fed and starved herring larvae is shown in Figure 1. Samples were taken in the range 1 to 9 days of starvation at several ages. With larvae older than 10 days, starved for 4 to 9 days, a significant decrease in tryptic enzyme activity can be observed compared with continuously fed larvae (independent *t*-test, $p < 0.05$). Since larval length is an easy to measure feature in field samples, but not age, tryptic enzyme activity is related to larval size for fed and starved herring larvae in Figure 2. An increase in tryptic enzyme activity with size in fed as well as in starving larvae was observed, but there was a significant overall difference between both groups (*t*-test, analyses of variance, $p < 0.05$). A linear regression analysis was fitted to the samples and shown with the 99% confidence limit. The confidence limit of the starving larvae was used as the range to evaluate the amount of starving larvae in the field samples (see Fig. 7a, b and Fig. 8).

The effect of different food concentrations on tryptic enzyme activity was demonstrated with laboratory-reared turbot larvae. Turbot larvae reared with a prey density of 2 *Brachionus* + 1 *Artemia* ml^{-1} (high density) are compared with larvae fed with 0.5 *Brachionus* + 0.1 *Artemia* ml^{-1} (low density) as well as with starved larvae (Fig. 3a) in relation to larval age after hatching. In larvae older than 10 days, significant differences in enzyme activity exist between larval batches reared on high food concentration and low food concentration as well as in starved larvae (independent *t*-test, $p < 0.05$). The difference between well-fed and starved larvae is much more pronounced in experiments with turbot than with herring larvae. Significant differences in enzyme activity were found for different food concentrations, but both fed groups showed very similar curves. The unexpected reduction in tryptic enzyme activity in larvae older than 23 days was suspected to be due to an infestation with parasites. Larvae were being treated but the mortality rate increased dramatically during this period. The reduced digestive enzyme activities indicated that feeding activity was much reduced under medical treatment.

Comparison of the growth rate (age/length relationship) showed deficiencies for the larvae kept on low food density (Fig. 3b). The length dependency of tryptic enzyme activity related to food density or deprivation is given in Figure 3c. Well-fed larvae showed a steep increase in enzyme activity with growth compared to the weak increase in these values for larvae kept on low feed density. Starved larvae, however, showed the lowest enzyme activities of the three conditions tested. Significant differences (*t*-test, analyses of variance, $p < 0.05$) exist between all three treatments, as shown for larvae > 5 mm (corresponding to larvae at 10 days of age and older).

The overall trends in the development of tryptic enzyme activity in well-fed laboratory-reared, herring,

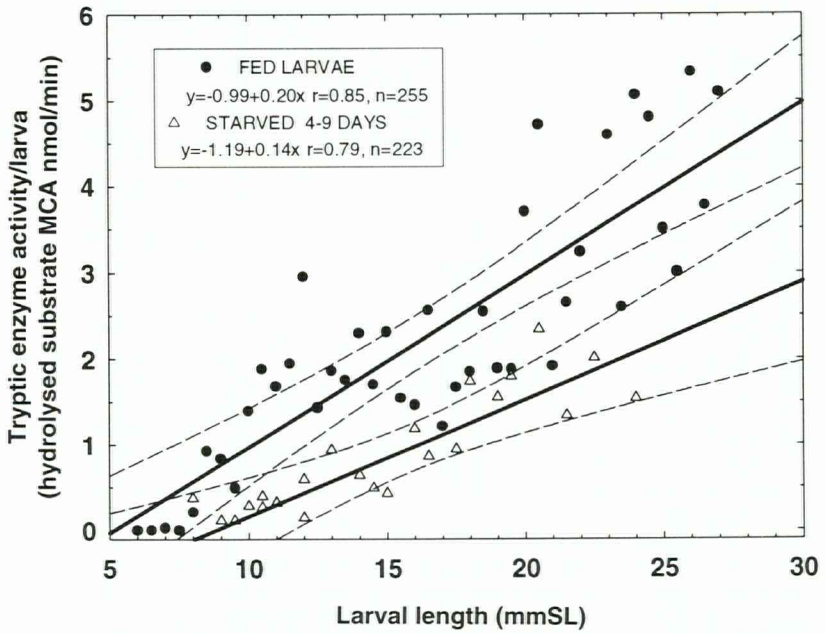


Figure 2. Tryptic enzyme activity of fed and starved herring larvae related to larval length. Data points are means of 5 to 15 individually measured larvae. Starved larvae were deprived of food for from 4 to 9 d. The means of the starving larvae are significantly different from the means of the fed larvae (*t*-test, analyses of variance, $p < 0.05$). Lines were fitted by linear regression analysis (equations and number of analysed larvae are noted in the graph) and the 99% confidence limit of the regression for 4 to 9 d starving larvae is shown; this confidence limit was applied in this study to evaluate the amount of starving herring and sprat larvae in field samples (see Figs. 7 and 8).

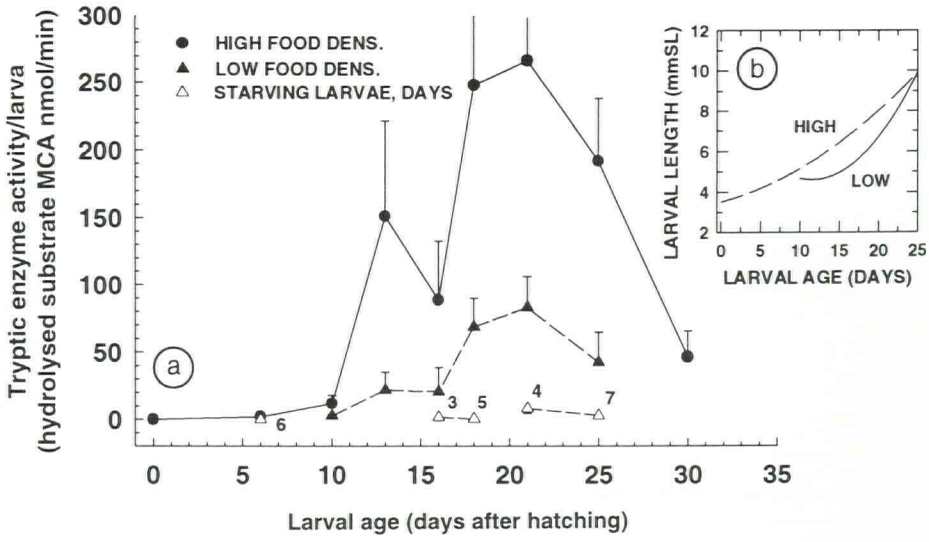
cod, and turbot larvae are shown in Figure 4 from hatching to an age of 18 days. The rate of increase in enzyme activity is very similar in herring and cod and not significantly different, but enzyme activities in turbot showed a very different trend and significantly higher values throughout the observed period (Tukey-Student-Newman-Keuls test, $p < 0.05$).

The diurnal pattern of tryptic enzyme activity in laboratory-reared herring larvae in relation to food uptake is shown in Figure 5a–c. Three different age groups (30, 37, 42 days after hatching) are compared. Feeding always resulted in an increase of tryptic enzyme activity, followed by a decrease in younger stages. The increase in the 40-day-old larvae persisted, indicating greater digestive enzyme capacity. Because of the size dependency of enzyme activity, mean length of the larvae for each sample was compared; no significant length differences between samples of the day were found (independent *t*-test, $p < 0.05$). Alterations in tryptic enzyme activity were not caused by differences in the mean length. The tryptic enzyme activity in the sample prior to the first-feeding of the day (pre-feeding level) was always above that of the starved larvae and more pronounced in older larvae.

Recovery of digestive enzyme capacity after a starvation period has been demonstrated in turbot larvae belonging in two different age groups (Fig. 6a, b). Larvae were deprived of feed for 2 or 3 days and re-fed again several hours prior to sampling. In both experiments the larvae showed reduced tryptic enzyme activity in the samples prior to feeding in the range expected for starving larvae of the same age (dotted lines). After food was supplied and larvae started to feed actively, enzyme activity increased within hours and persisted above the "starvation range" after about 24 h (pre-feeding level, Fig. 6a). Larval length (SL) was tested for significant differences in the mean length between the samples (independent *t*-test, $p < 0.05$), the statistical results indicated that differences in the activity could not be attributed to differences in mean length.

Field samples

A comparison of laboratory calibration experiments with known feeding levels and herring larvae sampled in the field with unknown condition is presented in Figure 7a, b. Samples from two different spawning sites (English Channel in January, Fig. 7a, and northern North Sea in October, Fig. 7b) are compared. Individual tryptic enzyme activities for larvae caught between 0600 and 0600 only are shown and compared with the regression fitted to tryptic enzyme activities of starving herring



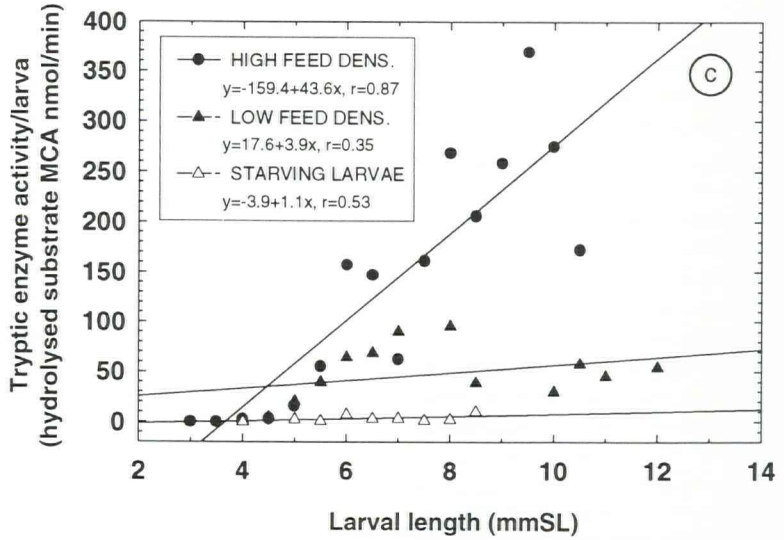


Figure 3. (a) Tryptic enzyme activity of laboratory-reared turbot larvae (*Scophthalmus maximus*) fed at two different levels (high food density: *Brachionus* 2 ml^{-1}, *Artemia* 1 ml^{-1}; low density: *Brachionus* 0.5 ml^{-1}, *Artemia* 0.1 ml^{-1}) and starved larvae (4 to 7 d) in relation to larval age. Data points with positive error bars (SD) are means of 5 to 15 individually measured larvae. Numbers indicate days the starving larvae were deprived of food prior to sampling. (b) Length–age relationship of the turbot larvae fed on two different food densities as shown in Figure 3a. (c) Tryptic enzyme activity of turbot larvae as shown in Figure 3a related to larval length. Data points are mean values of 5 to 15 individually measured larvae. The means of the fed larvae are significantly different from those of the larvae kept on low food density and the starving larvae (*t*-test, analyses of variance, $p < 0.05$).

larvae from the laboratory calibration data in relation to larval length (4 to 9 days starvation periods, see Fig. 2). The confidence limit (99%) of the regression was used as a limit to estimate the percentage of starving larvae in the field samples. In the sample of the English Channel, 9.1% of larvae were categorized as starving, whereas in the sample of autumn spawners no starving larvae were identified. The size distribution shows more larvae in the upper length range in the autumn sample, but even the smaller larvae from the northern North Sea were in better condition than the larvae from the English Channel in January.

A field study on sprat in the German Bight was established to examine the high variability in the recruitment processes of clupeiform species (under a SARP study, Sardine and Anchovy Recruitment Programme). Some of the investigations dealt with comparison of the condition of different larval cohorts throughout the whole

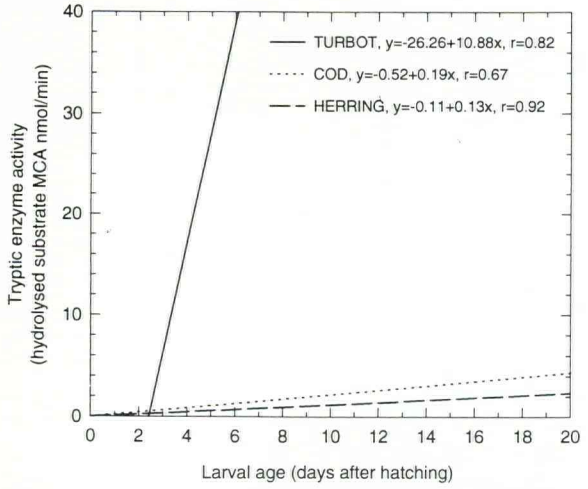


Figure 4. Comparison of mean tryptic enzyme activity development of laboratory-reared 1 to 18-d-old, cod, herring, and turbot larvae. Lines were fitted by linear regression analysis; equations and correlation coefficients are shown in the box.

major spawning season. Some of the results of this study are shown in Figure 8, depicting tryptic enzyme activity of sprat larvae individually measured and related to larval length. For these comparisons, larvae are not separated into day and night hauls; no clear trend was found between day and night samples with regression analyses (data not shown). The confidence limit (99%) of the linear regression analysis fitted to starving herring larvae from laboratory calibration experiments (Fig. 2) was used to evaluate the condition of sprat larvae in each sampling period. Assuming that starving sprat larvae would show similarly low tryptic enzyme activities as herring larvae, most of the starving larvae were found during the first two cruises in May and June: 34.4% and 27.9% respectively. The lowest number of starving larvae in terms of enzyme activity level were found during the third and fifth cruises in late June and July and in early August with only 5% and 3.4% of sprat larvae in comparatively poor condition. A size-specific comparison did not alter the percentage of sprat larvae in poor condition (data not shown).

The feeding rhythm of sardine larvae was determined in samples from a 48 h drift station off the Spanish northwest coast. Mean length and mean tryptic enzyme activity are shown (Fig. 9). Very large and very small larvae were excluded for this comparison in order to reduce the influence of larval length on tryptic enzyme activity pattern. No correlation was found between mean length and mean activities. Larval size was not of significant influence on the diel pattern of tryptic enzyme activity (independent t-test, $p < 0.05$). Two pronounced maxima and minima appeared during the 48-h period.

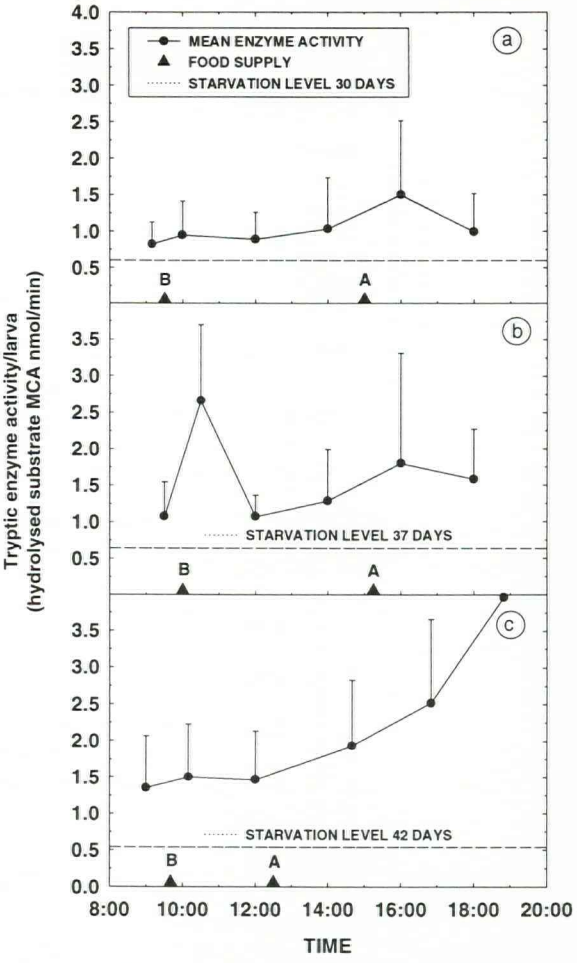


Figure 5a–c. Diurnal pattern of tryptic enzyme activity in relation to feed and feeding time in laboratory-reared and continuously fed herring larvae (*Clupea harengus* L.) aged 30 (a), 37 (b), and 42 (c) days after hatching. Arrows indicate the time when food was supplied (*Brachionus plicatilis* 5 ml^{-1}, *Artemia* nauplii 1 ml^{-1}). Data points with positive error bars (s.d.) are means of 10 to 15 individually measured larvae. The dotted line indicates the level of continuously starving herring larvae and their age is noted.

The first maximum was verified by the peak in the second 24 h. The difference in the maximum values was expected due to a known high individual variability in tryptic enzyme activity for fed larvae.

Discussion

The use of enzyme activity as an indicator for fish larval condition was examined in order to find an objective tool to describe the nutritional status of fish larvae. In an earlier study, tryptic enzyme activity was found to be influenced by the amount of food offered to fish larvae (Ueberschär, 1985).

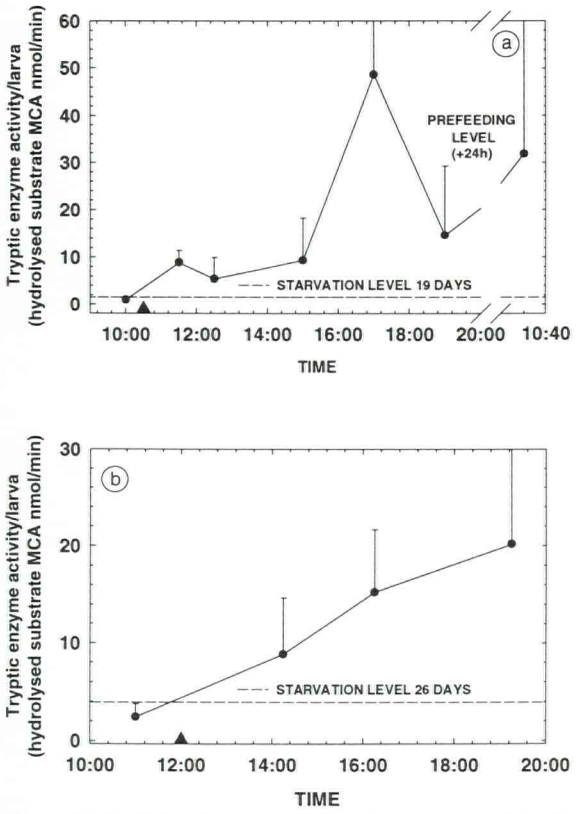


Figure 6a, b. Diurnal pattern of tryptic enzyme activity in relation to feeding time in laboratory-reared and subsequently starved turbot larvae (*Scophthalmus maximus*), aged 19 (a) and 26 (b) days after hatching. Arrows indicate the time when food was supplied (*Artemia* 1 ml^{-1}). Data points with positive error bars are means of 10 to 15 individually measured larvae. The dotted line indicates the level of continuously starving turbot larvae; their age is noted. The pre-feeding level refers to the tryptic enzyme activity of the larvae sampled 24 h later prior to first-feeding of the day.

Tryptic-like enzymes are widely occurring proteolytic enzymes. The occurrence of alkaline protease trypsin has been demonstrated in many marine organisms, including fish larvae (herring, cod, turbot, sandeel), invertebrates (*Crangon*, crab zoea, chaetognath, cephalopod larvae; Ueberschär, unpubl.) and some potential food organisms for fish larvae (*Acartia tonsa, Artemia* nauplii; Ueberschär, unpubl.). Consequently, there is the potential to use tryptic enzyme activity in answering similar questions (as dealt with in this study) in investigations on the nutritional condition of other organisms, for example copepod populations. However, it has been demonstrated that potential food organisms for fish larvae (copepod-nauplii or *Artemia* nauplii) have rather weak activities. Part of the tryptic enzyme activity measured in a fish larva with food in the gut may be attributed to exogenous trypsin, but only a minor part of the total enzyme activity will have its source in the ingested food organisms. The major part is synthesized by the larva itself as a reaction to physical stimulation due to food ingestion (Fänge and Grove, 1979; Hjelmeland *et al.*, 1988; Pedersen and Hjelmeland, 1988; Pedersen and Andersen, 1992).

The calibration of the indicator trypsin with larvae reared under controlled laboratory conditions is very important in the application of this methodical approach to any population of fish larvae with an unknown feeding history in order to determine their nutritional condition, even in field samples. At present, detailed calibration data are available for herring and turbot larvae as shown in this study. It is thought that each species exhibits its own characteristic tryptic enzyme activity level in relation to its current nutritional condition. Herring larvae deprived of food reacted first with a slight decrease in enzyme activity but starving for a longer period resulted in significant differences when compared with continuously fed larvae. Additionally, a pronounced age and length dependency in relation to enzyme activity was detected, which has to be taken into account when applying these baseline values to field samples. Compared to herring larvae, turbot larvae respond to food deprivation with a much more pronounced and faster decrease in tryptic enzyme activity. The absolute values for tryptic enzyme activity in fed turbot larvae are also much higher than in herring. The values found for starving herring larvae (as shown in Fig. 2 and used in this study for comparison with herring and sprat larvae sampled from the field) could not be applied to turbot larvae from the field, for instance. The development of mean tryptic enzyme activity in fed larvae of cod, herring, and turbot with age is shown in Figure 4, indicating significant differences between turbot and the other two species and can be an indication of differences in functional development of the digestive tract in fish larvae. Success in larval turbot rearing in aquaculture may be attributed in part to the rapid development of their digestive capacity, which is expressed by the high level of proteolytic enzyme activity, as found with trypsin and pepsin (Ueberschär, 1993).

Further aspects of the application of tryptic enzyme activity measurements concern the quantity, quality, and time of feeding in aquaculture. With turbot larvae, it was shown that food density influences the amount of tryptic enzyme activity per larva and ultimately the growth rate. Permanently inadequate food supply may result in reduced growth rate, an important aspect for successful aquaculture. In laboratory calibration experiments, it was shown that feeding time influences the amount of tryptic enzyme activity measured. From the practical point of view, in laboratory and field research, time of sampling should be taken into account when measurements of tryptic enzyme activity are used to

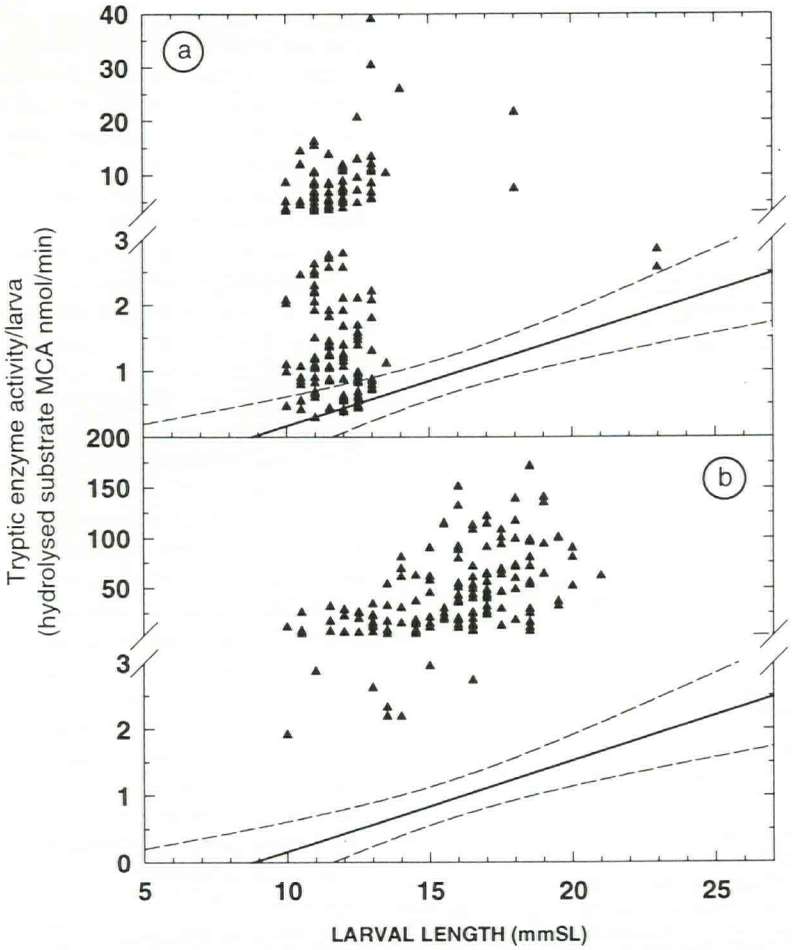


Figure 7a, b. Estimation of the percentage of starving herring larvae (*Clupea harengus* L.) in field samples from the English Channel (7a) and a drift station northwest of Scotland (7b) using the calibration data of laboratory-reared larvae. Data points represent larvae individually measured. The "starvation range" used is described in Figure 2. ▲ = individuals.

evaluate larval condition. In aquaculture, continuous individual monitoring of larval tryptic enzyme activity can give indications about feeding activity, feeding success, and the optimal size of the feed ration which should be offered to a certain species. The results could be related to quantity and quality of food supplied and possibly certain other environmental factors. Infestation with parasites in experiments with turbot larvae resulted in reduced feeding activity and consequently in decreasing tryptic enzyme activity levels. Therefore tryptic enzyme activity measurements could be useful in detecting unfavourable health conditions.

In field investigations on larval fitness it is important to know how the physiology of fish larvae responds to subsequent poor feeding conditions. Experiments with turbot larvae deprived of food for 2 to 3 days showed no deficiencies in response to re-feeding conditions. When fed, a re-establishment of enzyme activity was observed without any noticeable delay with a pronounced increase compared to continuously fed turbot larvae. The response is certainly species and age dependent, but nevertheless the methodological approach can generally be used to determine experimentally the "point of no return" with different starvation or feeding periods.

The exact amount of starving herring larvae in the field sample can be determined by using the calibration data from laboratory-reared herring larvae. The example given compares larval cohorts from autumn and winter spawners. It was expected that larvae hatched in the autumn would have a higher probability of survival. Potentially more appropriate food resources are available at that time compared with the situation in the late winter season. The results confirm this assumption. With the autumn samples, no starving larvae were detected, whereas in the sample of winter spawners 9.1% larvae were in poor condition. Furthermore,

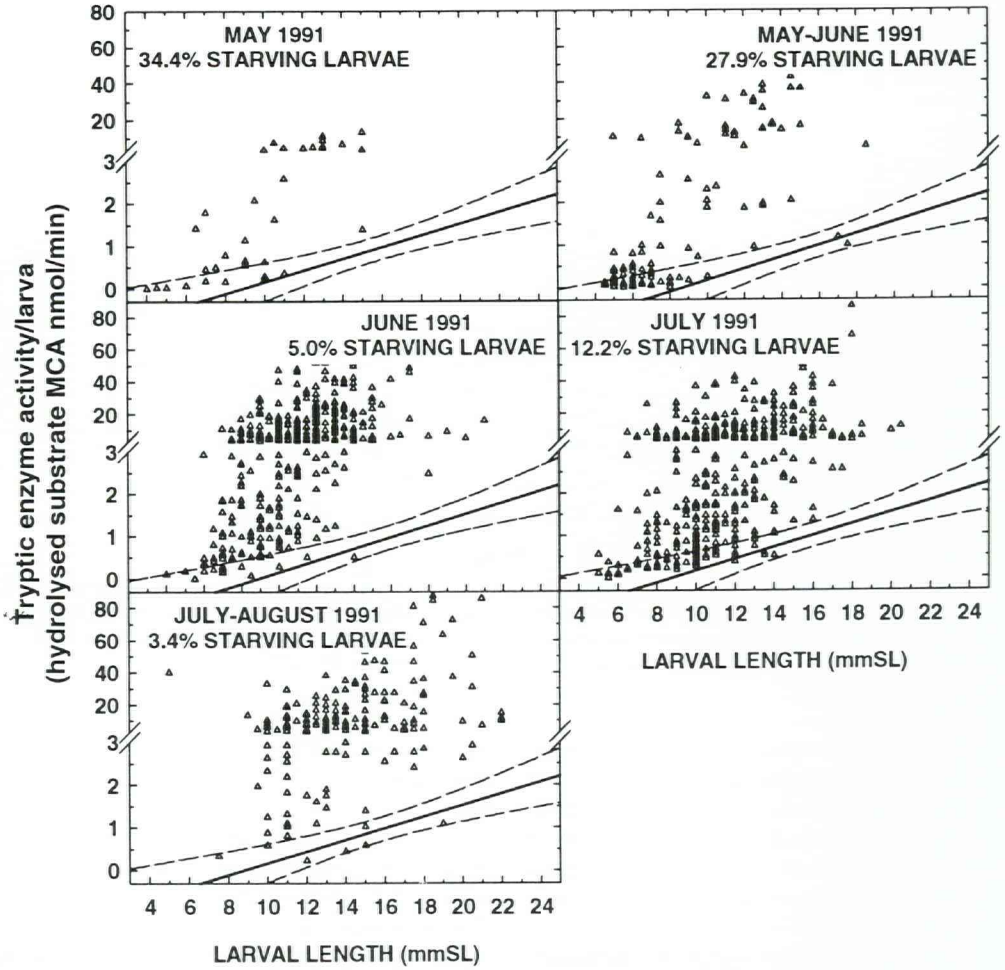


Figure 8a–e. Estimation of the percentage of starving sprat larvae (*Sprattus sprattus*) sampled during a field study in the North Sea from May to August 1991. The 99% confidence limit of the linear regression analysis fitted to the means of starving herring larvae from laboratory calibration served as the "starvation range" to evaluate the number of starving sprat larvae (see Fig. 2).

herring larvae from field samples showed, in the main, higher trypsin levels than fed laboratory-reared intervals. This can be explained by the different food quality. It must be assumed that wild larvae mostly feed on food of optimal quality, whereas cultured larvae are given food of questionable quality. Commonly used rotifers and even enriched *Artemia* nauplii still cannot substitute for natural plankton. These results indicate the value of digestive enzyme activity measurements when food quality is being investigated.

In a field study, calibration data from herring were applied to sprat larvae sampled throughout the whole major spawning season in 1991 in the German Bight. This study was aimed at the recruitment variability of clupeiform species and the condition analysis is intended to give information about favourable or unfavourable conditions for the sprat larvae at a certain time. The surviving juveniles can be counted and their hatching date can be "back-calculated" by otolith reading. High survival rates should be reflected in fewer starving larvae for the corresponding hatching period. Analysis of survival rates of the juveniles, condition data, potential zooplankton abundance, and abiotic factors (hydrography) can result in new information on the conditions which are particularly favourable for larval survival in the field. The application of herring calibration data to sprat larvae from field investigations has uncertainties in the prediction of starvation, but will not have a strong influence on the relative relationship when different larval cohorts are compared. The results presented in this study identified two favourable periods and this should be reflected by high survival rates of the juveniles from the related hatching period (data are still being processed and will be presented in future publications).

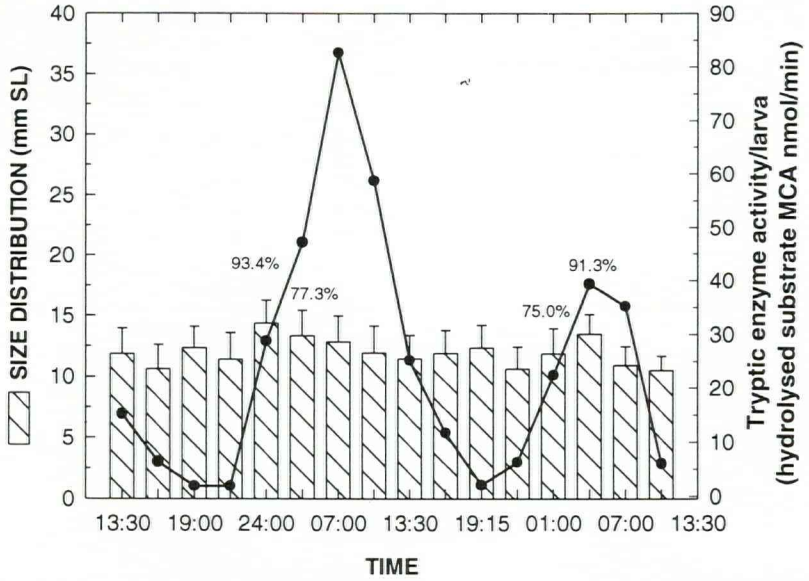


Figure 9. Diurnal pattern of tryptic enzyme activity levels for sardine larvae (*Sardina pilchardus*) sampled at a 48-h drift station in the Atlantic Ocean off the Spanish northwest coast. Data points are means from 10 to 18 sardine larvae individually measured. The bars with positive error bars (SD) give the mean length distribution per sample. Percentages of larvae with inflated gas bladders are indicated on the mean length bar of the corresponding sample. □ = Mean length, ● = mean activity/larva.

As shown by the experimental results of this study, fish larvae have a natural diurnal rhythm in tryptic enzyme activity and the feeding rhythm of sardine larvae sampled on a 48 h drift station (Fig. 9) was determined in order to show their diurnal feeding pattern. No significant differences in the mean length distribution were found; diurnal pattern in tryptic enzyme activity is therefore likely to be a reaction to feed ingestion. Oscillations of tryptic enzyme activity can be regarded as a consequence of periodic food ingestion. The main feeding time is supposed to be at dusk. Main digestion periods are indicated by the highest enzyme activity values, which were found from midnight until the early morning. As shown with the diurnal pattern of continuously-fed herring larvae for instance, the lower pre-feeding level did not drop below the level for starving larvae. Consequently the minimum in enzyme activity does not indicate starving sardine larvae. The proposed feeding time is confirmed by observations about the volume of the gas bladder as found in analysed sardine larvae. In four of the 16 samples taken on the drift station, a high percentage of larvae with inflated gas bladders were observed during the night (between midnight and 0330). With an inflated gas bladder, larvae find swimming activity difficult, and it is assumed that they are unable to feed during this period. The background for the periodic inflation of the gas bladder is not yet clear (Hoss *et al.*, 1989). One reason for this behaviour could be that neutral buoyancy allows the larvae to "rest" in order to conserve energy which can be invested in digestion processes. Additionally, in order to inflate the gas bladder, the larvae must migrate to the surface, where, in most areas, surface temperature is higher than in the deeper layers. Higher temperatures increase the rate of biochemical reactions and metabolic processes in poikilotherms (RGT-rule), and digestion would be enhanced by this behaviour.

Summarizing the results presented in this study, the application of tryptic enzyme activity measurement on field samples can serve to determine relative differences in the number of larvae in poor condition, and this approach can be applied to aquaculture, for instance when different feeding regimes are employed or in open systems such as net cages, where larvae are fed with natural plankton. Periodic monitoring of larval condition can give an indication of the survival of larvae and can indicate causes of year-to-year variability.

References

Bailey, K. M., and Houde, E. D. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. mar. Biol., 25: 1–83.

Fänge, R., and Grove, D. 1979. Digestion. *In* Fish physiology, vol. 8, pp. 161–260. Ed. by W. S. Hoar, D. J. Radall, and J. R. Brett. Academic Press, New York.

Hjelmeland, K., Huse, I., Jørgensen, T., Molvik, G., and Raa, J. 1984. Trypsin and trypsinogen as indices of growth and survival potential of cod (*Gadus morhua* L.) larvae, pp. 189–211. *In* The propagation of cod, *Gadus morhua*, Part 1. Ed. by E. Dahl, D. S. Danielssen, E. Moksness, and P.

Solemdal, Institute of Marine Research, Flødevigen Biological Station.

Hjelmeland, K., Pedersen, B. H., and Nielsen, E. M. 1988. Trypsin content in intestines of herring larvae, *Clupea harengus* ingesting inert polystyrene spheres or live crustacea prey. Mar. Biol., 98: 331–335.

Hoss, D. E., Checkley, D. M., and Settle, L. R. 1989. Diurnal buoyancy changes in larval Atlantic menhaden (*Brevoortia tyrannus*). Rapp. P.-v. Réun. Cons. int. Explor. Mer, 191: 105–111.

Houde, E. D. 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp., 2, 17–29.

Hunter, J. R. 1976. Report of a colloquium on larval fish mortality studies and their relation to fisheries research, Jan. 1975. NOAA Tech. Rep. NMSF Circ., 395. 5 pp.

Lauff, M., and Hofer, R. 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. Aquaculture, 37: 335–346.

Pedersen, B. H., and Andersen, K. P., 1992. Induction of trypsinogen secretion in herring larvae (*Clupea harengus*). Mar. Biol., 112: 559–565.

Pedersen, B. H., and Hjelmeland, K., 1988. Fate of trypsin and assimilation efficiency of larval herring (*Clupea harengus* L) following digesting copepod nauplii. Mar. Biol., 97: 467–476.

Pedersen, B. H., Nilssen, E. M., and Hjelmeland, K. 1987. Variations in the content of trypsin and trypsinogen in larval herring (*Clupea harengus* L) digesting copepod nauplii. Mar. Biol., 94: 171–181.

Pedersen, B. H., Ugelstad, I., and Hjelmeland, K. 1990. Effects of a transitory, low food supply in the early life of larval herring (*Clupea harengus*) on mortality, growth and digestive capacity. Mar. Biol., 107: 61–66.

Ueberschär, B. 1985. Untersuchungen über die Verdauungsenzyme von Fischlarven. Dipl. Thesis, Institut für Meereskunde, Univ. Kiel. 102 pp.

Ueberschär, B. 1988. Determination of the nutritional condition of individual marine fish larvae by analyzing their proteolytic enzyme activities with a highly sensitive fluorescence technique. Meeresforsch, 32: 144–154.

Ueberschär, B. 1993. Measurement of proteolytic enzyme activity: significance and application in larval fish research. *In* Physiological and biochemical aspects of fish development. Ed. by B. T. Walther and H. J. Fyhn. University of Bergen.

Ueberschär, B., and Clemmesen, C. 1992. A comparison of the nutritional condition of herring larvae as determined by two biochemical methods – tryptic enzyme activity and RNA/DNA ratio measurement. ICES J. mar. Sci., 49: 245–249.

Ueberschär, B., Pedersen, B. H., and Hjelmeland, K. 1992. Quantification of trypsin with a radioimmunoassay in herring larvae (*Clupea harengus* L.) compared with a highly sensitive fluorescence technique to determine tryptic enzyme activity. Mar. Biol., 113: 469–473.

Wiebe, P. H., Burt, K. H., Boyd, S. H., and Morton, A. W. 1976. A multiple opening/closing net and environment sensing system for sampling zooplankton. J. mar. Res., 34: 313–326.